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NUTRITION REVIEWS

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STATUS OF IRRADIATION PRESERVATION OF FOODS

Radiation has been conceived as accomplishing several results in connection with preservation. The attainment of these results requires the use of particular amounts of radiation. A knowledge of what is required for each purpose is significant not only in accomplishing the purpose but also in establishing the degree of other changes, for example, flavor changes, resulting from the radiation treatment. Hence, such knowledge is basic to all other considerations.

Radiation of foods is measured in terms of the radiation energy absorbed by the food. The unit of measurement is the "rad", which is defined as the energy absorption of 100 ergs per gram, independent of the nature of the irradiated substance.

The most radiation-resistant bacterium of public health significance is *Clostridium botulinum*. While the concept has been accepted that the radiation dose required to destroy this organism will vary with the food in question, the current working figure for complete destruction of this organism is 4.8 million rad. This is a large amount of radiation.

The dose requirement for pasteurization varies greatly with the food item and with the desired product life extension. In all probability, most pasteurizing applications will fall within the range of 50,000 to 500,000 rad. The dose required to produce sexual sterilization (not death) for *Trichinae* in pork is considered to be about 30,000 rad.

For sprout inhibition in tuberous vegetables the dose appears to be of the order of 7,000 rad, and is fairly critical since other changes affecting storage stability are produced by larger amounts of radiation.

Changes induced by radiation vary greatly with the food item in question and with the amount of radiation employed. Of the changes involved in food acceptability from an organoleptic point of view, the following may be listed: flavor change (unique, but resembling scorching); color changes (both darkening and bleaching); textural changes (especially noticed with fruits and vegetables); and changes on storage of foods resulting from action of agents not controlled by radiation, including enzyme reactions and chemical reactions.

To give a complete picture would require an individual discussion of each food item. However, it may be noted that, at sterilizing doses, flavor changes are virtually non-existent in certain sea food items such as oysters and shrimp. Likewise, chicken and pork display hardly detectable off-flavors. Beef, on the other hand, ordinarily develops a strong off-flavor. Recent work at the University of Michigan indicates that certain combinations, either natural or synthetic, of materials found in tomato will control the development of off-flavor in beef. Dairy products, in general, do not react favorably to sterilization by radiation. Work at M.I.T. is under way on a process of concurrent radiation and vacuum deodorization. This process shows some promise.

At pasteurizing doses, many products have acceptable flavors, including most meats, sea foods, and certain fruits and vegetables. In particular instances, there may be other changes induced by the radiation which will affect the acceptability of the product. This is especially true in meats where chemical changes lead to appearance degradation in a short time. It would appear that there may be some useful applications of pasteurization in the treatment of certain fruits and vegetables such as citrus fruits.

The level of radiation required to control *Trichinae* in pork does not affect its acceptability. Likewise, the level of radiation proposed for control of sprouting has no effect on acceptability of potatoes and onions.

In all foods which contain naturally occurring enzymes, radiation at the sterilizing level and below does not inactivate such enzymes. The amount of radiation which will inactivate these enzymes appears to be of the order of several times that required for sterilization. Hence, it is present thinking that some other process for inactivating enzymes will be needed. At this time, the most effective method seems to be heat inactivation. Thus, one may conclude that foods which contain enzymes will first be heat-treated and consequently will have some cooked characteristics. Failure to inactivate the enzyme leads to flavor changes, texture changes and, in certain instances, the growth of tyrosine crystals through hydrolysis of the protein (in meat for example).

Product acceptability has been evaluated largely by scientifically trained people. There has been some exposure to consumer-type groups in academic circles and in certain military establishments. Perhaps a fair summary of the results to date would be as follows.

Even with strong irradiated flavors, an inexpert group on first exposure will not detect them. Practically all testing with inexpert groups has involved single exposures; hence, there is no current knowledge as to what will happen on replication. Work with expert panels indicates that ability to detect irradiated flavor can be improved through practice, and once a sensitivity to it has been developed, the flavor becomes objectionable. What is needed are additional consumer studies involving replications. It is probable that the Army Quartermaster will conduct such studies in the near future.

A great deal of effort has been expended both with private and Army funds on investigating the wholesomeness of irradiated foods. Much of this work has been planned by highly competent groups, including the Federal Food and Drug Administration, the Surgeon General of the Army, and various academic and industrial organizations. The findings to date have been almost exclusively favorable. Studies involving short- and long-term animal feedings, enzyme system studies, carcinogenic activity, and limited human feeding have pointed to no real difficulty attributable to changes caused by irradiation. One investigator has observed a hemorrhagic syndrome in rats fed irradiated beef. Current thinking is that this observation has no significance for the wholesomeness of beef.

Currently, there is considerable concern in the area of induced radioactivity in irradiated foods. When certain forms of ionizing radiation are employed, certain atoms present in the food become radioactive. These effects occur principally with the use of high voltage electron beams, although they will result from the employment of gamma ray sources, which include some neutron flux. The level of radioactivity induced is low but measurable. One might hold the view that the level is so low as to have no significance when one considers other sources of radioactive atoms normal to the human diet, or when one considers exposures to ionizing radiation normally encountered from various sources such as therapeutic and diagnostic x-rays and cosmic rays. However, this subject is under active consideration by the Surgeon General of the Army, the Army Quartermaster, and the Federal Food and Drug Administration.

One can understand the viewpoint that holds objection to any added ionizing radiation exposure. To indicate how seriously this matter is being considered, the

Army recently prohibited the consumption of any irradiated food by any of its personnel.

The significant forms of radiation for preservation are electron beams and gamma rays. There have been available for a number of years various sources of electron beams, the most significant of which are the Van de Graeff, the resonant transformer, and the linear accelerator. The first two sources appear to be the least expensive; however, they cannot produce electron beams beyond 5 million volts. The linear accelerator theoretically has no top limit, and the Army Ionizing Radiation Center at Lathrop, California, is to be equipped with a 24 million volt linear accelerator. This will provide a penetration of about six inches of unit density materials.

Gamma ray sources are principally of two kinds: (1) the fission products from uranium or plutonium reactors; or (2) artifically made radioactive materials such as cobalt 60. The gamma rays usually have a penetration of a foot or more. The fission products can be used as purified materials, such as cesium 137 or "hot" fuel rods taken directly out of a reactor. It is probable that the Army Ionizing Radiation Center will be equipped with a cobalt 60 gamma ray source.

While there is considerable difference of opinion on this point, it would appear that the electron beam sources are more economical than the gamma sources at this time.

One might analyze the work to date, as far as food items are concerned, as largely directed to proving wholesomeness and finding out which items are acceptable organoleptically. There has been some basic research to find ways to control the flavor changes resulting from radiation. So far, this has not been very fruitful. The notable exception seems to be the work at the University of Michigan where, apparently, materials have been found which block the chemical reactions involved in the flavor change. It is clear that much more basic research needs to be done in order to establish radiation preservation as a workable food process.

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TRACE ELEMENTS IN ENAMEL AND DENTIN

Ancient teeth from excavations contained concentrations of various trace elements very similar to those in teeth from contemporary individuals. Teeth from individuals in the Orient and from the Arctic Circle of Canada contained high fluoride levels.

Increasing information is becoming available on the minute composition of enamel and dentin. The greatest volume of information pertains to the variations in concentration of the fluoride ion in proportion to the amount of fluoride ingested during tooth development (*Nutrition Reviews* **10,** *28 (1952)*). Fluoride has also been shown to have an interesting gradient from very high levels on the surface of the enamel, decreasing rapidly to relatively low levels in the internal enamel (*Ibid.* **14,** *230 (1956)*). In addition, a wide variety of other trace elements has been found to be present in enamel and dentin (*Ibid.* **14,** *230 (1956)*).

In an investigation to compare the trace element content of modern teeth with those of ancient teeth, L. T. Steadman *et al.* (*J. Dent. Res.* **38,** *285 (1959)*) conducted analyses for carbonate, fluoride, zinc, silicon, manganese, lead, silver, copper, tin,

strontium, iron and aluminum. Twelve Pueblo Indian permanent teeth buried in Pueblo Bonito, Mexico, for approximately eight hundred years, and 23 Indian Knoll permanent teeth excavated from the bank of Green River in Kentucky after an interment of approximately five thousand years, were analyzed. The analyses were made on successive layers of the enamel and the roots, which had been prepared by grinding over a porcelain filter.

In the Pueblo Indian teeth all the trace elements studied occurred in greater concentrations in the outer than in the inner enamel, whereas the carbonate concentration increased from 1.75 per cent in the outer layer to a maximum of 2.44 per cent in the deepest layer of enamel. In the external enamel, fluoride at 1,640 p.p.m. occurred in the greatest concentration, followed by zinc, silicon, manganese, lead, silver, copper and tin at 1.0 p.p.m. The concentration of zinc varied less from the outer to the inner surface of the enamel than for any of the other trace elements. In root dentin of these same teeth, the various trace elements again occurred in greater concentration in the surface than in the subsurface layers, with the exception of manganese, for which a fairly uniform distribution was noted.

In the Indian Knoll teeth, the distribution of trace elements was generally similar to that in the Pueblo Indian teeth. However, the enamel surface accumulation of fluoride, silicon and manganese was more marked than among the Pueblo Indian samples. Moreover, the Pueblo Indian teeth contained greater amounts of zinc, silicon, manganese and silver but less copper than the Indian Knoll teeth. Also, the root dentin in the Pueblo Indian teeth contained much greater concentrations of fluoride, zinc, silicon and manganese than enamel, but this ratio was much less for the Indian Knoll teeth.

It is of interest that the distribution of these trace elements in contemporary teeth was found to be remarkably similar to that in these ancient Indian teeth. This finding was contrary to expectation and indicated that a much higher percentage of trace elements had been incorporated into teeth during life from the foods consumed than was acquired during centuries of interment. This should serve as a warning signal to those who have sought to date the age of exhumed teeth on the basis of trace element composition. Fluoride especially has been considered as a reliable index for this purpose, with high fluoride levels being considered indicative of long burial and low fluoride levels of short intervals.

In bone where there is increased permeability to soil waters as the organic matrix is removed, the ability to use fluoride content as an index of length of burial may have greater validity than for teeth. However, even in the case of bones, the level of fluoride in the bone at the time of death varies tremendously, as do the amounts and fluoride levels of soil water.

The extent to which fluoride concentrations in the enamel and dentin vary in human populations is just beginning to be realized. J. H. Shaw, O. P. Gupta and M. E. Meyer (*Am. J. Clin. Nutrition* **4**, *246* (*1956*)) have reported data on the fluoride concentrations of teeth from citizens of Bombay and Delhi, India, and from Boston, U.S.A. In all three communities, the fluoride content of the water supplies was low. In 28 individuals from Delhi, the average fluoride levels of the enamel and dentin were 299 and 710 p.p.m., respectively. The 36 subjects from Bombay had average fluoride levels in the enamel and dentin of 184 and 456 p.p.m., respectively, while the values for 18 Bostonians were 58 and 109 p.p.m., respectively.

In a later survey, J. H. Shaw, J. B. Resnick and E. A. Sweeney (*J. Dent. Res.* **38**, *129* (*1959*)) reported further information on individuals from Boston, U.S.A.; Delhi, Calcutta, Bombay, Lucknow, Dibrugarh, India; and from five different

groups of Indians and Eskimos in the Canadian Arctic. Smaller numbers of teeth were available from Lahore, Pakistan, and from an army base in Iran. The lowest levels of fluoride were observed in the enamel and dentin from Boston, averaging 54 p.p.m. and 86 p.p.m., respectively. Although there were extremely wide variations in fluoride values within each group, all other population groups had significantly higher fluoride contents than the Boston group, with the highest content in Iran averaging 267 p.p.m. for enamel and 911 p.p.m. for dentin while Delhi averaged 261 p.p.m. for enamel and 730 p.p.m. for dentin.

The average fluoride levels for the enamel and dentin of Eskimo teeth were appreciably higher than the Boston values. The primitive Eskimos from either a coastal culture or a caribou culture, and the Eskimos residing in the neighborhood of trading posts, as well as the primitive Indians, had average fluoride values varying in the enamel from 164 to 185 p.p.m. and in the dentin from 471 to 596 p.p.m. However, the Indians residing in the neighborhood of trading posts had appreciably lower levels than the preceding four groups, with averages of 118 and 309 p.p.m., respectively, for enamel and dentin.

These data suggest that there are relatively large areas of the world where significant amounts of fluoride are available for human consumption, judging by the amount incorporated into tooth structures. The levels observed in the course of this survey were sufficiently high to give major benefits in the control of tooth decay.

GASTROINTESTINAL PROTEIN LOSS AND HYPOPROTEINEMIA

A large molecular polymer labeled with I^{131} has been used in a group of patients to study loss of protein from the gastrointestinal tract.

The numerous causes of hypoproteinemia have been reviewed earlier (*Nutrition Reviews* **15,** *232 (1957)*). In one type of hypoproteinemia termed idiopathic or essential, the usual causes for the low serum protein levels have been eliminated. Inanition has been ruled out; in fact, many of the patients had been on high protein diets. Neither external losses of protein through the skin or kidneys have been demonstrated nor have increased losses of nitrogen in the stool been observed.

Studies using I^{131}-labeled albumin in measuring turnover rates suggested that the deficiency in plasma protein in these patients was related to the increased rate of protein degradation rather than to an error in protein synthesis. Thus, the term "hypercatabolic hypoproteinemia" has been used by some to designate this type of disorder. While the total plasma proteins are low, the major decrease is confined to the albumin fraction.

Y. Citrin, K. Sterling and J. A. Halsted (*New Engl. J. Med.* **257,** *906 (1957)*) studied intensively a patient who had giant hypertrophy of the gastric mucosa, a condition which is associated with hypoproteinemia and edema. From the mucosal surface of the stomach, there was an oozing of protein-containing fluids. In studying the albumin turnover rates in this patient using I^{131}-labeled albumin, they noted a rapid turnover rate of a small albumin pool. Fluid removed from the stomach during the study contained large amounts of the tagged albumin. It has been suggested that the albumin turnover rates for this patient were almost identical to those reported by earlier investigators in patients with "hypercatabolic hypoproteinemia." The flow of albumin into the lumen of the stomach accounted

for the accelerated loss of activity (I^{131}-labeled albumin) from the plasma.

The use of iodinated albumin for the detection of leakage from the blood stream into the stomach or upper intestinal tract has the inherent difficulty that albumin is digested by proteolytic enzymes and the resulting amino acids, including the labeled ones, are absorbed and degradated in the body. Thus, any tagged material excreted into the gastrointestinal tract is, in turn, further degradated and may not be detectable in the feces.

Recently, R. S. Gordon, Jr., (*J. Polym. Sci.* **31,** *191 (1958)*) found that I^{131}-labeled polyvinylpyrrolidone (P.V.P.), a large molecular weight polymer, could be used to determine whether there was a defect in the intestinal mucosa which would allow large particles to pass readily from the blood stream into the lumen of the stomach or intestine. Since the P.V.P. is not attacked by the proteolytic enzymes present in the intestine, it was a suitable indicator for determining leakage into the gastrointestinal tract, and tagging it with I^{131} made its detection easier.

R. S. Gordon, Jr., (*Lancet* **I,** *325 (1959)*) has recently reported in preliminary form the results of the administration of the labeled P.V.P. to nine patients who were suspected of having increased loss of the protein from the gastrointestinal tract. In carrying out the tests, each subject received intravenously 10 to 25 microcuries of the tagged material and feces were collected for 96 hours and pooled as a single sample. After homogenization, the radioactivity in an aliquot of the feces was measured. In a series of 41 control subjects who had no disease or who were hospitalized because of disorders not affecting the gastrointestinal tract or the metabolism of plasma protein, it was found that from 0 to 1.5 per cent of the administered P.V.P. was excreted in the feces.

The presence of hypoproteinemia per se did not influence the results, since six patients with hypoproteinemia attributed to hepatic disease and three with hypoproteinemia associated with malignancy were studied, and the amount of radioactivity in the feces was similar to that found in the control group (0 to 1.5 per cent).

In a series of nine patients with "hypercatabolic hypoproteinemia" it was found that between about 2.5 and 20 per cent of the administered activity appeared in the feces. In all cases there was also an abnormally rapid degradation of administered isotopically labelled albumin.

In the same issue of *Lancet*, M. Schwartz and S. Jarnum (*Ibid.* **I,** *327 (1959)*) reported studies in four patients who had idiopathic hypoproteinemia. They also used iodinated P.V.P. which was obtained from Dr. Gordon. They noted that a patient with hypertrophic gastritis lost nine times the amount of iodinated P.V.P. in the feces as did a normal individual who was studied at the same time. In all four patients with idiopathic hypoproteinemia, the amount of P.V.P. found in the stool following intravenous injections of the tagged material was abnormally large, indicating a leaking of the colloids from the blood stream into the stomach or intestinal lumen.

It has been suggested that the term "hypercatabolic hypoproteinemia" is not applicable in these cases. They are producing albumin at a rate which is close to the liver's maximum ability, but they are losing it into the stomach or intestinal lumen faster than it can be fabricated. Also, from a study of a single case, Schwartz and Jarnum suggest only tentatively that perhaps the hypoproteinemia commonly associated with sprue is the result of protein loss into the intestine rather than malabsorption as has generally been assumed.

The possibility of increased loss of protein through the intestinal tract in other disorders, such as ulcerative colitis, will probably be studied using this technique. The

cause of the defect in the intestinal mucosa has not been elucidated and microscopic examination has not as yet given definitive information. However, it has been postulated that an increased capillary permeability of the mucosa exists in this condition.

Gordon has suggested that since to date no case with the clinical features of idiopathic "hypercatabolic hypoproteinemia" has shown a normal radioactive P.V.P. excretion test, perhaps it may be correct to implicate the gastrointestinal tract as a route of loss of protein from the plasma in all of these cases. Also, since the loss of protein into the stomach or intestine has metabolic implications similar to loss by way of the urine or the skin, he has suggested that the term "exudative enteropathy" be used in such cases and reserve the term "hypercatabolic hypoproteinemia" for patients in whom a true exaggeration of endogenous metabolism has been determined.

FIBRINOLYSIS AND LIPID METABOLISM

Patients with abnormal lipid metabolism were compared with normal controls both before and after a fatty meal to determine whether the coagulability and fibrinolytic activity of the blood was affected by hyperlipemia.

The possibility that atherosclerosis is initiated or progresses by means of a thrombus of fibrin forming on the vessel wall has been proposed by J. B. Duguid (*J. Path. Bact.* **58,** *207 (1946)*). In fact, T. Astrup (*Lancet* **II,** *565 (1956)*) and A. L. Copley (*Ibid.* **I,** *102 (1957)*) have postulated that a delicate equilibrium between fibrinolysis and coagulation may exist at all times. The possibility that hyperlipemia may accelerate blood clotting while at the same time retarding fibrinolysis has been proposed by H. W. Fullerton (*Brit. Med. J.* **1,** *663 (1955)*) and H. B. W. Greig (*Ibid.* **2,** *708 (1957)*).

Fibrinolytic activity or clotting has usually been evaluated either in normal subjects after eating a fatty meal or by the addition of fat to plasma *in vitro*. One exception is the study by E. Sohar, M. C. Rosenthal and D. Adlersberg (*Am. J. Clin. Path.* **27,** *503 (1957)*), in which patients with abnormal lipid metabolism were the subjects.

In order to get information about the relationship between hyperlipemia, increased coagulability of blood and heart disease, S. I. Nitzberg, M. A. Peyman, R. Goldstein and S. Proger (*Circulation* **19,** *676 (1959)*) have made a study of the blood fibrinolytic activity in a group of patients with idiopathic hyperlipemia and primary hypercholesteremia both before and after a fatty meal.

Three groups of subjects were selected. Group one included nine patients with idiopathic hyperlipemia, a disorder of fat metabolism in which the serum is milky and contains high concentrations of triglycerides even in the fasting state. Elevation of cholesterol or phospholipid may or may not be present. Evidence of ischemic heart disease was present in some cases.

The second group consisted of ten patients with primary hypercholesteremia. The serum of this type of patient is clear and contains high levels of cholesterol, even in the fasting state. Serum phospholipid is also usually elevated. Half of the cases had xanthomatous lesions and, of these, three had ischemic heart disease.

"Normal" subjects comprised the third group. The selection was based on (1) relative youth, (2) serum that was clear with "normal" cholesterol and triglyceride levels, (3) absence of xanthomatous lesions, and (4) no indication of ischemic heart disease.

The subjects fasted from 10:00 p.m. until 9:00 a.m. when blood was drawn and they were fed a fatty meal.

Four hours after the fatty meal, blood was drawn using a siliconized needle and a multiple syringe technique. At first, blood was taken for the determination of serum lipids. Next, a siliconized 30 ml. syringe was used and a stop watch started when the blood entered the syringe. Finally, 1.5 ml. of blood were drawn in a siliconized tuberculin syringe for thromboelastography and a second stop watch was started.

Several tests of the clotting mechanism were employed. Included were determinations of prothrombin concentration of plasma, factor V and factor VII activity, plasma fibrinogen level, coagulation time of recalcified plasma, whole blood clotting time in silicon glassware, prothrombin consumption of plasma and serum, heparin tolerance test, thromboelastography, the thromboplastin generation test, the one-stage prothrombin time and the Stypven time.

Two methods were used to measure the fibrinolytic system; one measured fibrinolysis and the other measured profibrinolysin activity of plasma. Although the interpretation of results was more difficult in the latter test, the error was much less between duplicate samples. Therefore there was more certainty concerning any significant change in fibrinolysis time.

The results of the coagulation studies may be classified into two main categories. The first includes specific coagulation factors such as fibrinogen or prothrombin. There were no obvious differences in any of these values in the three groups, either after fasting or following a fatty meal.

In category two, the over-all coagulability of the blood was determined either with or without the addition of thromboplastic material. All tests performed without the addition of thromboplastic material were essentially within normal limits in all subjects before and after the fatty meal.

In the determination of Stypven time, in which Russel viper venom serves as a thromboplastic agent, acceleration of coagulation occurred in the fasting hyperlipemic patients and in all groups following the fatty meal. As might be anticipated, the degree of shortening of the Stypven time after the meal was less in the hyperlipemic and hypercholesteremic subjects than in the controls. Only one patient in the first group and two in the second group showed a decrease of more than four seconds, whereas seven out of ten control subjects showed more than four seconds shortening of the Stypven response.

The fact that the Stypven time was always decreased in fasting patients with idiopathic hyperlipemia indicated that the higher levels of triglycerides might be responsible. In the majority of cases, however, no strict correlation could be seen between Stypven time and level of triglycerides.

Similarly, in all three groups after the fatty meal there was elevation of triglycerides and shortening of Stypven time, but no strict correlation could be demonstrated when the results in individual patients were analyzed.

No correlation was observed between Stypven time and serum cholesterol, phospholipid or beta-lipoprotein either during fasting or after a fatty meal.

The fibrinolysin test showed great variation between individuals in the fasting state. Following the fatty meal all individuals except one in each group showed acceleration of fibrinolysis time.

Measurement of profibrinolysin activity showed almost identical times in the fasting normal and hypercholesteremic groups. The hyperlipemic group had a prolonged streptokinase-induced fibrinolysis time. After the fatty meal, most individuals showed prolongation of the streptokinase-induced fibrinolysis.

The fact that no association was noted between Stypven time and the level of the combined serum phospholipids in this

series does not necessarily exclude the phospholipids from having a role in altering blood coagulability. J. R. O'Brien (*Lancet* **I,** *1213* (*1957*)) observed that Stypven time was decreased after eating small quantities of phospholipids, even though there was no increase in the level of serum phospholipid.

He postulated that phospholipid which is inactive in the test tube, becomes activated in the body so that Stypven time is accelerated. He suggested that an increase in some single phosphatide could be responsible for the shortening of the Stypven time. Another possibility is that only certain fatty acids in the phospholipid esters are active in producing this effect.

Summary: Three groups of subjects, including patients with idiopathic hyperlipemia, patients with hypercholesteremia and normal subjects, were given tests of clotting or fibrinolytic activity of the blood before and after a meal containing fat.

Values for specific coagulation factors were normal in all groups both before and after the fatty meal. The measure of over-all coagulability gave normal results in most cases. The exceptions were the thromboplastin generation test and the Stypven time. The Stypven time was shortened in the patients with hyperlipemia even when fasting, and in all three groups after the fatty meal. The results of the thromboplastin generation test were provocative but inconclusive.

Direct measure of blood fibrinolysis gave inconclusive results but the measure of profibrinolytic activity indicated prolongation associated with serum triglyceride levels and turbidity.

The fact that these abnormalities in the fluidity of the blood are only demonstrable when indirect methods are used, suggests that powerful protective mechanisms are at work to counteract the effects of fatty meals and high blood lipids upon the clotting and clot dissolving mechanisms. It is difficult to see how the results lend much support to the Duguid hypothesis of atherogenesis.

PLASMA CLEARING EFFECT OF GASTRIC MUCIN

Feeding of gastric mucin caused a decrease in the optical density of plasma of normal and atherosclerotic subjects, and prevented the postprandial lipemia in anginal patients.

The clearing of lipemic plasma by heparin (*Nutrition Reviews* **12,** *294* (*1954*)) and other compounds is of current and practical interest in so far as these substances may influence transport and metabolism or tissue deposition of lipids (*Ibid* **13,** *20* (*1955*)). Other sulfated polysaccharides have been studied in relation to experimental atherosclerosis (*Ibid* **15,** *187* (*1957*)). Likewise gastric mucin has been shown to clear lipemic plasma, as has an acid aminopolysaccharide isolated from hog gastric mucin that has been shown to be chemically similar to hyaluronic acid, without anticoagulant action, and effective orally.

B. Rossi and V. Rulli have reported on the plasma-clearing effect of gastric mucin in healthy and atherosclerotic subjects under basal conditions (*Circulation* **18,** *397* (*1958*)). The authors studied five healthy human subjects (mean age 33 years) and ten (mean age 62 years) with atherosclerosis as evidenced by angina pectoris or previous myocardial infarction. The optical density of plasma was determined on five specimens drawn hourly in the fasting state, but showed no significant change. The same determinations were made after 250 mg. of gastric mucin had been given orally to each of these same subjects. Only average results from each group were reported, but the authors state that plasma optical dens-

ity always dropped after gastric mucin was given with the maximum percentage decrease occurring at three or four hours. The same plasma samples after 20 hours at room temperature showed no significant changes in optical density in either group.

The two groups of subjects were not at all comparable in age, and the plasma optical density was considerably greater in the older atherosclerotic group than in the younger normal subjects. Nevertheless, plasma clearing had evidently occurred in each group after oral administration of gastric mucin.

The authors point out the similarities and differences between the effect of heparin and gastric mucin. They doubt that the clearing power of mucin is "due to an enzymatic effect of the lipoprotein lipase type such as the one that is apparently responsible for the heparin clearing effect." Evidence pro or con is not adduced, however.

In the article immediately following the above (*Circulation* **18,** *400 (1958)*), Rulli and Rossi report on plasma turbidity changes and electrocardiographic alterations induced by alimentary hyperlipemia in anginal patients before and after the administration of gastric mucin. Ten patients were selected for typical angina pectoris with evidence of coronary insufficiency and ECG changes after a fat meal. They were given a fat meal consisting of 200 g. of homogenized 40 per cent cream. Plasma optical density was determined hourly for five hours and serial electrocardiograms were recorded. The patients were then retested, receiving 250 mg. gastric mucin with the fat meal. The hyperlipemia and (in half of the cases) the ECG changes were prevented.

A number of questions are raised by the two papers of Rossi and Rulli. First, as the authors themselves point out, gastric mucin has various actions, including the binding of vitamin B_{12}, growth factor activity for a *Lactobacillus* species, antianemic action and protection against fatty liver. With such a variety of biologic effects from even a more or less purified preparation, one has difficulty drawing clear conclusions. Does mucin bind and so hinder the absorption of some compound; or does it facilitate the digestion and absorption of some substance with quite the opposite effect? The area for speculation is wide indeed.

As to the electrocardiographic changes attributed to lipemia, the evidence is far from definite. The alterations are minor (reduced T-wave voltage with biphasic or inverted T-waves) and inconstant. The two records reproduced show almost flat T-waves before the test meal, biphasic in some leads. The changes after the fat meal may well be non-specific postprandial effects associated with the cardiovascular changes due to digestion rather than to lipemia per se.

Thus, though there are many uncontrolled factors which make it difficult to conclude what mechanisms are involved, the papers of Rossi and Rulli are of interest in demonstrating the lipemia-clearing effect of feeding mucin and the prevention of lipemia after a fat meal.

URINARY FLUORIDE EXCRETION IN CHILDHOOD AND PREGNANCY

Studies conducted in Jerusalem where the water contains 0.6 p.p.m. fluoride indicated that women in the second and third trimesters of pregnancy and young children excreted lower concentrations of fluoride in the urine than nonpregnant adults.

A relatively large body of information has been collected on the influence of varying sources and amounts of fluorides on urinary excretion (E. J. Largent, *Metabolism of Inorganic Fluorides.* Chapter from *Fluoridation as a Public Health Measure*, edited by

J. H. Shaw. American Association for the Advancement of Science, Washington, D. C. (1954)). In general, the data on urinary excretion for human beings has been limited to age groups in the high school or adult range with practically no data for children.

F. J. McClure and C. A. Kinser (*Pub. Health Reports* **59,** *1575 (1944)*) noted a strong correlation between the concentration of fluoride that was naturally present in the drinking water and the concentration excreted in the urine of high school boys. For 173 boys in Aurora, Illinois, where the drinking water contained 1.0 p.p.m. fluoride, the urinary fluoride excretion varied from 0.7 to 1.1 p.p.m., while in Galesburg, Illinois, where the drinking water contained 1.9 p.p.m. fluoride, the urinary excretion of fluoride varied from 1.2 to 2.2 p.p.m.

Although there are definite disadvantages in reporting fluoride excretion in terms of concentration rather than in total fluoride excretion per day, I. Zipkin, R. C. Likins, F. J. McClure and A. C. Steere (*Pub. Health Reports* **71,** *767 (1956)*) have reported that urinary fluoride concentration does not vary greatly in relation to the time of day at which the specimen is collected.

I. Gdalia (*J. Dent. Res.* **37,** *601 (1958)*) has reported the results of fluoride determinations on urinary samples collected from 59 small children (one to six years old) and 34 adults (20 to 55 years old) residing in Jerusalem, where the water supply naturally contains 0.5 to 0.6 p.p.m. fluorides. Since it proved to be impractical to collect 24-hour urine samples, one to four samples were taken from each person between 9:00 a.m. and 3:00 p.m. on different days. This sampling procedure was believed to be adequate since different samples from the same person on different days agreed within the error of the method used.

The mean urinary fluoride concentration of the 59 children was 0.2 p.p.m. whereas the value for the 34 adults was 0.52 p.p.m. A further age difference was noted in that the 31 children between one and three years of age had an average urinary fluoride excretion of 0.14 p.p.m., while the value for the 28 children between four and six years of age was 0.27 p.p.m. The difference between the fluoride concentrations for the two groups of children was judged to be statistically significant at the 1 per cent level.

The author concluded that the difference between the fluoride excretion concentrations for children and adults was attributable to a greater fluoride storage in the skeletal tissues of children. This greater rate of deposition in the bones of children may be caused by the rapid growth and calcification of the bones during childhood. However in the present study, there are no data on the total amount of fluoride ingested nor the total amount excreted by the individuals under investigation. In view of this lack, the actual amount being stored cannot be determined.

This study may indicate only that adults ingest a relatively higher amount of fluoride daily than do children since they consume more water, more beverages made from water and more foods cooked in water. Children, on the other hand, take in a higher percentage of their water in the form of low-fluoride foods such as milk, raw fruits and vegetables.

An interesting observation on fluoride metabolism during different stages of pregnancy has been reported by J. Gedalia, A. Brzezinski and B. Bercovici (*J. Dent. Res.* **38,** *548 (1959)*). Samples of urine voided between 9:00 a.m. and noon were obtained from a total of 196 healthy pregnant women who, like the children in the previous study, resided in Jerusalem. Samples were first collected in the fourth month of pregnancy and then at approximately monthly intervals until two or three months after delivery. The highest number of cases tested at any one stage was 117 in the fourth month, and the lowest number was 55 at one month

after delivery. The collection of 24-hour urine samples was not practicable so data on total daily excretion of fluoride were not available. Nor were data collected on total daily consumption of fluoride.

The mean urinary concentration of fluoride at the fourth month of pregnancy was 0.53 p.p.m. The successive mean values for each month from the fifth to the eighth were significantly lower than for each preceding month until a low of 0.22 p.p.m. was observed in the eighth month. The mean value in the ninth month was 0.29, which represented a significant increase over the value for the eighth month. At one month and two or three months after delivery, the urinary fluoride concentrations were 0.39 and 0.49 p.p.m., respectively. The mean fluoride urinary concentration for adult males and for nonpregnant adult females was 0.52 p.p.m. Hence the post-pregnancy urinary fluoride concentration did not return to the pre-pregnancy level until two or three months after delivery.

These data suggest that there is a greater retention of fluorides during pregnancy, and for a brief period thereafter, than there is in the nonpregnant adult female or the adult male. The average decrease in urinary fluoride concentration during the last six months of pregnancy appears to be in the neighborhood of 0.2 p.p.m. If the average amount of fluoride consumed daily is assumed to be constant before and during pregnancy, and if the average urinary output is assumed to be one liter per day, then approximately 0.2 mg. more fluoride was retained per day during the last two-thirds of pregnancy, or a total of about 35 mg. of fluoride during the 180 day period.

It would be extremely interesting to know how this amount was distributed among the several possible sites; the maternal skeleton, the placenta and embryonic membranes and the fetus. Attention should also be drawn to the fact that this accumulation is not major and definitely is not to be considered in the range of potential toxicity.

As in the study with children, the full interpretation of these data from pregnant women cannot be made without values for dietary intake and total excretion. However, both these studies suggest that fluoride storage in children and pregnant women differs from that in adult males and nonpregnant females. More precise data would answer the interesting questions that have been raised by the above reports.

AMINO ACID AVAILABILITY IN MAN

Availabilities of ten amino acids from pork, peanut butter, egg and milk, milk and cottage cheese proteins are compared to those of egg protein during studies in male college students.

The biological availability of essential amino acids, as well as the essential amino acid composition, is an important factor in assessing the biological value of a protein. J. H. Watts *et al.* (*J. Nutrition* **67,** *483, 497 (1959)*) have determined the availability in male college students of the eight amino acids essential for human adults, and of cystine and tyrosine in diets containing whole egg, pork muscle and peanut butter (first study) and whole egg, egg and milk, milk alone and cottage cheese (second study). Because of its high biological value, egg protein was included as a reference protein to which the other test foods could be compared. Cystine and tyrosine were studied because of their sparing action on methionine and phenylalanine, respectively.

Caloric intakes required to maintain body weights and protein intakes required to maintain nitrogen equilibriums were es-

tablished in trial periods. Experimental periods were of ten days duration. Urine and fecal collections were made for six days, following a four day adjustment period. Carmine was used as the fecal marker.

Each series of studies on test proteins was preceded by feeding of a vegetarian diet containing 0.8 to 0.9 g. nitrogen and sufficient calorie adjustor to provide between 43 and 48 calories per kg. of body weight per day. Subsequent protein containing diets were made isocaloric and of similar carbohydrate to fat distribution by changing the amount of calorie adjustor. Protein containing diets were all isonitrogenous with the reference egg protein diet. Vitamins and minerals were added as needed to make the diets conform to the National Research Council allowances.

Daily urine collections were preserved with 2 per cent acetic acid, while fecal specimens were frozen daily and six day composites homogenized and the homogenate autoclaved and subsequently dried by lyophilization. Urine was analyzed for total nitrogen and creatinine, and food and feces were also analyzed for total nitrogen and assayed microbiologically for amino acids.

The per cent availability of amino acids in the total diet, making no allowance for fecal amino acids of endogenous or bacterial origin, and the per cent availability of amino acids in each of the test foods were calculated as follows:

The per cent availability in the total diet equalled the quotient × 100 of the intake from total diet less the amount excreted in feces divided by the intake from total diet; the per cent availability in the test food was the quotient × 100 of the intake from test food less the difference between fecal excretions during the test food and low nitrogen periods divided by the intake from test food. Since amino acid excretions in the feces were frequently less during periods when protein was fed than during the low nitrogen period, the availability values of test foods alone, as calculated, often exceeded 100 per cent.

The authors have inferred that the amount of nutrient excreted varied with the type of diet and hence that the calculation of availability of test foods alone lacked significance. They suggest that the large excretions of amino acids on the low nitrogen diet are of endogenous or bacterial origin.

Availability data for total diets revealed similarities for egg, egg and milk combined, and pork diets. Availabilities of amino acids in the egg protein reference diets differed between the first and second studies, being greater for each amino acid during the egg-pork-peanut butter study than during the egg-milk-cottage cheese study. Interindividual variability was greater among subjects of the latter study, suggesting that availability depends not only on the protein and the type of diet but also on the characteristics of the individual under study.

The availabilities of the amino acids in the peanut butter diet were consistently lower than the availabilities in the egg or pork diets, except for the availability of tyrosine. The availabilities of the amino acids in the milk diet were less than 5 per cent lower than the corresponding egg diet except for cystine which was as much as 25 per cent lower. The egg and milk diet compared favorably with the egg diet alone, while the cottage cheese diet also compared favorably except for cystine, whose availability was 10 per cent below that in the egg and the egg and milk diets.

Nitrogen balance data indicated the least retention during the peanut butter diet of the first study, a phenomenon which the authors attributed to the low available methionine and cystine values in the diet. In the second study, nitrogen balances were less positive during the milk and cottage cheese diets than during the egg and egg and milk diets. The authors have suggested that the low intakes of available methionine

and cystine were again responsible for the results observed. High fecal nitrogen values on the milk diet implied low digestibility of large quantities of milk, and high urinary nitrogen values on the cottage cheese diet implied the presence of a limiting nutrient.

The data generally indicate that the availabilities of amino acids as calculated for total diets containing protein are, at the very least, 75 per cent and usually exceed 85 per cent, while rarely if ever exceeding 95 per cent.

Studies of amino acid availability are of interest because of its relationship to protein synthesis. Protein synthesis depends on the presence simultaneously of a complete mixture of essential amino acids; hence the effective composition of a food protein is more important than its total composition (*Nutrition Reviews* **5,** *144 (1947)*). This effective composition is influenced by the rate of digestion of the protein, the presence of digestive enzyme inhibitors (*Ibid.* **5,** *338 (1947)*), amino acid antagonism, the absorption rates of individual amino acids and the simultaneous appearance of essential and nonessential amino acids in suitable proportions at the site of synthesis.

These studies were planned to test the digestibility (availability) of individual amino acids fed as complete proteins. True digestibility could not be computed because, in certain instances, fecal excretions were less during protein feeding periods than during the low nitrogen diet periods.

Hence these studies have provided information only on gross availability by the measurement of amino acids in food and feces. They do not provide data on the resistance of various proteins to hydrolysis by digestive enzymes, the possible presence of enzyme inhibitors, absorption rates of individual amino acids or amino acid antagonism; and information on protein synthesis is given only through evaluations of nitrogen balance. Furthermore, they do not elaborate the detailed mechanisms underlying amino acid availability in man.

These studies do, however, provide gross information about five specific diets in a large number of human subjects. The tables quantifying fecal amino acid excretion are in themselves valuable contributions. Nonetheless, because of the differences in amino acid composition of the various diets and in amount of calorie adjustor added, and because of the unknown influences of bacterial and endogenous synthesis of amino acids, even the gross implications of the data must be viewed as tentative. Extension of these studies in man to include work with purified amino acids and isotopically labelled amino acid tracers would be received with interest.

REPRODUCTION UNDER THE STRESS OF PHOSPHORUS DEFICIENCY

Phosphorus deficiency in pregnant ewes leads to lowered plasma phosphorus levels and a marked decrease in the weight and ash content of the bones.

A study has been reported of the effect of mild phosphorus deficiency on the gestating and lactating ewe by D. Benzie *et al.* (*J. Agr. Sci.* **52,** *1 (1959)*). This is a sequel to an earlier study of calcium deficiency carried out under similar conditions (*Nutrition Reviews* **15,** *249 (1957)*).

Sixty ewes, all three and one-half years old and in early pregnancy, were divided into six experimental treatments. Group A was slaughtered immediately for carcass analysis and the remainder was housed in separate indoor pens. Each animal in groups D, E and F was then offered each day 100 g. of chopped oat straw and 800 g. of a concentrate mixture (dried sugar-beet pulp 79 per

cent, corn gluten meal 10 per cent, corn meal 8 per cent, and blood meal, meat meal and sodium chloride all 1 per cent). During late pregnancy the concentrate ration was increased to 1000 g., and during lactation to 1400 g. so as to provide levels of protein and energy close to those recommended for these conditions (National Research Council, *Recommended Nutrient Allowances for Sheep*, 1945).

The concentrate proved to be unpalatable during late pregnancy and the formula was then changed to include an extra seven parts of corn and two parts of corn gluten meal at the expense of beet pulp.

The rations were designed to provide 1.0 g. phosphorus per day at first, rising to 1.9 g. per day in mid-lactation, and falling again to 1.1 g. after lactation had ceased. The corresponding levels of calcium were 5.4, 8.8 and 5.4 g. per day. The levels of phosphorus were expected to be inadequate, but the levels of calcium were those which had proved nutritionally adequate in the earlier study (*Nutrition Reviews, loc. cit.*).

The animals in groups B and C were offered the same rations together with a daily supplement of disodium hydrogen phosphate containing 3.0 g. phosphorus to bring the level up to the standard recommendations.

Failure of appetite is a common symptom of phosphorus deficiency which can upset the interpretation of experiments. However, with the change in formula of the concentrates and the occasional use of potatoes and turnips (which provided no more than 1 per cent of the total dry matter intake) as succulents to stimulate appetite, more than 90 per cent of the rations were eaten in every group.

After approximately 120 days on the experiment the ewes had their lambs. At the usual reckoning period of four weeks of age, both the supplemented ewes (groups B and C), and the non-supplemented ewes (groups D, E and F) had an average of 1.8 surviving lambs each. The mean weight of the lambs was then approximately 20 pounds in each case.

At mid-lactation (60 days after the birth of the lambs) the non-supplemented ewes weighed 115 pounds, and those having received the phosphate supplement had a mean weight of 129 pounds. One group of each type was then killed and dissected for analysis of the skeleton. The mean ash content of the skeletons from group D (non-supplemented) was only 60 per cent of the weight of ash (1490 g.) found in the skeletons of the control group A killed at the beginning of the trial. The corresponding figure for group B (supplemented) was 82 per cent that of the controls.

The remaining groups continued to receive their experimental rations for a further 120 days until the "mid-dry period," but one of the previously unsupplemented groups (F) was given an additonal 3 g. of phosphorus per day in this period. The seven surviving ewes from the unsupplemented group E then had a mean live weight of 124 pounds as compared with 143 pounds for group C, which had received the phosphorus supplement throughout, and 128 pounds for group F, which had received it only in the final period.

All the ewes were then killed. At this point the skeletal ash weight of the supplemented group C was only 3 per cent below that of the original controls, a non-significant difference in the statistical analysis of the results. On the other hand, the ash in the non-supplemented group E, though higher than that for the corresponding group killed at the mid-lactation point, was still 23 per cent below that of the control group. Group F, which had received the late supplementation, was a little richer in its bone mineral content but also approximately 17 per cent below the original control figure.

As in the earlier study of calcium deficiency (D. Benzie *et al.*, *J. Agr. Sci.* **48**, *175 (1956)*) there were great differences in the extent to which individual bones in the

skeleton appeared to give up their mineral matter. The lower jaw, skull and cervical vertebrae seemed again to be the most sensitive to erosion. The shafts of the long bones were the least sensitive, although, significantly, those of group D had less ash than those of group A.

The changes in total weight of the bones corresponded largely to the changes in ash weight, although there were small decreases in the percentage ash content of the eroded bones. For example, the lower jaws from group D had 65.3 per cent ash content compared with a figure of 68.6 per cent for group A, while the corresponding figures for total ash content were 43.2 and 25.8 g., respectively.

Radiographs were taken of the live animals at intervals, and also of the flesh-free bones after the ewes were slaughtered. Although the radius and tibia were not among the most affected bones (as judged by changes in ash content), they showed the clearest differences in radiographs taken with the live animal, presumably because less definition was lost than with bones embedded more deeply in tissues and under a thicker coat of wool.

Grading of these radiographs by eye, on the basis that resorption was accompanied by a thinning of the cortex and the appearance of longitudinal striations, gave results in close agreement with those based on ash weight. With flesh-free materials the same relationship was confirmed for the lower jaw and cervical vertebrae.

Analyses for calcium and phosphorus were made from blood samples drawn from groups C and E at intervals of approximately 25 days throughout the experiment. The mean values for total serum calcium were approximately 10 mg. per 100 ml. throughout the trial for the supplemented group C, and 1 to 2 mg. per 100 ml. higher for the phosphorus-deficient group E, a result consistent with the necessarily greater mobilization of calcium from the bone during the resorption observed in the latter group.

The level of inorganic phosphorus in the whole blood from group C was approximately 6 mg. per 100 ml. until mid-lactation, from which time it rose slowly to about 9 mg. per 100 ml. In the deficient group E it began at 4 mg. per 100 ml., then decreased slowly to a value of only 1 mg. per 100 ml. at parturition; it did not rise again to its original value until after the lambs had been weaned. It appears, therefore, that the measure of blood phosphorus can serve as an indicator of an uncomplicated phosphorus deficiency under these conditions.

In general, as judged by the ash content of the skeleton, the recovery of the ewes receiving 4.5 g. phosphorus per day from the stress of a reproductive cycle (despite the indication of significant bone resorption during lactation) is in line with the accepted view that such resorption is a physiological phenomenon. On the other hand, failure to recover skeletal weight before the onset of the next reproductive cycle, as seen in the ewes receiving 1.5 g. phosphorus per day, is a true indication of a dietary inadequacy.

LATHYRISM—AN ANCIENT DISEASE; ODORATISM—AN EXPERIMENTAL MODEL

A water soluble toxic substance from sweet peas affecting mesenchymal tissue produces lesions in rats similar to defects occurring spontaneously in humans—suggestive of lathyrism, an ancient form of food poisoning.

Frequently, events occurring in the past have a far reaching influence upon modern progress. Studying them may furnish clues which excite the imagination, provide useful analogies, or serve to recall forgotten ideas. Such may be the case with lathyrism,

a form of food poisoning known and feared since the days of Hippocrates. D. K. Dastur (*Nutrition Reviews* **17,** *33* (*1959*)) recounted these historical events in a summary of this strange illness. Certain legumes, pulses and vetches (mostly of the *Lathyrus* family) grow in abundance throughout the world. Being hardier than domesticated crops, they can survive conditions which are ruinous to plantings of grain. Naturally, in times of famine, the people of the world have resorted to these legumes as their principal source of food, but with disastrous results.

However, these observations were chiefly of historical interest until I. V. Ponseti and W. A. Baird (*Am. J. Path.* **28,** *1059* (*1952*)) found that rats fed a diet containing 50 per cent *Lathyrus odoratus* (sweet pea) factor, developed not only scoliosis (reported previously by B. J. Geiger, H. Steenbock and H. T. Parsons (*J. Nutrition* **6,** *427* (*1933*)), but also aneurysms of the thoracic aorta.

Further work by Ponseti and R. S. Shepard (*J. Bone Joint Surg.* **36,** *1031* (*1954*)) demonstrated a startling similarity between the lesions produced in rats fed *L. odoratus* and defects occurring spontaneously in humans. This toxic substance which affected mesenchymal tissues adversely could simulate the pathologic lesions found in idiopathic (juvenile) scoliosis, adolescent kyphosis, intervertebral disc degeneration, spondylolisthesis, recurrent dislocation of the shoulder, Osgood-Schlatter's disease, degenerative arthritis, Paget's disease and dissecting aneurysms of the aorta. While the authors hastened to explain that they did not believe that odoratism produces these lesions in man, they did feel that some defect of mesodermal metabolism might be shared in common.

T. E. Bachhuber and J. J. Lalich (*Science* **120,** *712* (*1954*)) reported confirmation of the occurrence of aneurysms in rats fed *L. odoratus*.

Actually the term "lathyrism" should refer to the neurologic lesion, a degenerative process of the spinal cord which occurs in animals or men fed one of the related legumes (*Lathyrus, Vicia, Pisum, Ervum*), and "odoratism" should be used to describe the skeletal and vascular lesions induced in animals by *L. odoratus* (H. Selye, *Revue Canadienne de Biologie* **16,** *1* (*1957*); *Nutrition Reviews* **17,** *33* (*1959*)).

The water-soluble toxic factor was isolated by H. P. Dupuy and J. G. Lee (*J. Am. Pharm. Assn.* **43,** *61* (*1954*)). It was found to contain beta-(gamma-L-glutamyl)-aminopropionitrile (E. D. Schilling and F. M. Strong, *J. Am. Chem. Soc.* **76,** *2848* (*1954*)), and later the active compound was identified by Ponseti's group at Iowa (*Science* **121,** *63* (*1955*)) to be beta-aminopropionitrile (BAPN).

W. B. Bean was impressed by the similarity between the lesions found in congenital arachnodactyly (Marfan's Syndrome) and those described by Ponseti. Accordingly, these two made a study of the incidence of skeletal deformities in patients who had died from dissecting aortic aneurysms (Bean and Ponseti, *Proc. Second World Congress Cardiology* **2,** *389* (*1954*); *Circulation* **12,** *185* (*1955*)). Skeletal deformities were found in 35 per cent of autopsied patients who had aneurysms, but it occurred in less than 2 per cent of 600 hypertensive patients (100 by autopsy and 500 by clinical examination).

These observations, conjectural though they may have been, led to a search for factors which might identify the nature of the mesenchymal defect and which (hopefully) may correct it or halt its progress. Among the many substances studied, some failed repeatedly and others seemed to prevent lathyrism or odoratism, but the results could not be confirmed. These substances included casein, gelatin, methionine, cysteine, glutamine, vitamin A, thiamine, vitamin B_{12}, choline, ascorbic acid, vitamin D, vitamin E and pantothenic acid.

Selye reported (*loc. sit.*) that stress, immaturity or somatotrophic hormone accentuated the lesions produced by BAPN.

However ACTH, cortisone or thyroid hormone would lessen the extent of the lesions.

Ponseti has reported a continuation of his studies of thyroid hormone, triiodothyronine, and its acetic and propionic analogues (*Endocrinology* **64,** *795* (*1959*)). While each had some effect in ameliorating the lesions produced by BAPN, the effectiveness of each paralleled its ability to increase metabolic rate. Triiodothyronine continues to be the most effective form of prophylaxis yet studied.

Perhaps future clues may be found, such as the discovery of excessive amounts of hydroxyproline in the urine of patients with Marfan's Syndrome (A. Sjoerdsma, J. D. Davidson, S. Udenfriend and Z. Mitoma, *Lancet* II, *994* (*1958*); *Nutrition Reviews* **17,** *79* (*1959*)), or the finding that androsterone, which was produced more rapidly in patients given triiodothyronine, can alter lipid metabolism (L. Hellman *et al.*, *J. Clin. Invest.* **38,** *1010* (*1949*)).

APPETITE AND GROWTH STIMULATION BY TUMOR DIETS IN THE TUMOR-BEARING RAT

An increased salt intake may not be the sole explanation for the improvement in food consumption and growth of tumor-bearing rats fed diets containing a variety of tumor tissue.

Anorexia followed by weight loss is a common phenomenon among patient or animal hosts bearing clinical or experimental malignant neoplasms. C. Moreschi (*Z. Immunitätsforsch* **2,** *651* (*1909*)) proposed that the death of a tumor-bearing animal was due to the withdrawal by the neoplasm of a specific autogenous essential nutrient. C. P. Richter's work (*Harvey Lectures* **38,** *63* (*1942–1943*)) on self-selection of diets included observations that tumor-bearing rats often ate the cancers from their pen mates or even their own tumors.

Sprague-Dawley rats bearing transplanted Walker carcinoma 256 when maintained on a 25 per cent casein diet show an abrupt decrease in food intake and weight within 18 to 32 days of tumor implantation. When such rats are shifted to a diet containing lyophilized Walker carcinoma 256 (isocaloric with and containing an amount of nitrogen equal to that in the casein diet), they resume eating and gain weight. The tumor-fed rats show less carcass weight loss and grow larger tumors than the casein-fed rats. Death eventually overtakes both, although tumor ulceration and infection take so heavy a toll that longevity data for the two groups are not precise (F. K. Millar, J. White, R. H. Brooks and G. B. Mider, *J. Nat. Cancer Inst.* **19,** *957* (*1957*); G. B. Mider, *Proc. Canad. Cancer Conf.* **1,** *120* (*1955*)).

Studies of the growth and pattern of nitrogen excretion in normal rats fed similar casein and tumor-containing diets (J. White *et al.*, *J. Nat. Cancer Inst.* **19,** *969* (*1957*)) revealed that growth of the tumor-fed rats was equal to that of the casein-fed controls, whether the tumor tissue in the tumor diet equalled the casein content of the control diet in either total weight or in total nitrogen and calories, and whether the controls ate either ad libitum or were pair fed.

In the pair feeding experiments, the patterns of urinary nitrogen excretion revealed the following: (1) tumor-fed rats excreted less urea nitrogen than their casein-fed controls; (2) their allantoin nitrogen excretion exceeded that of their controls. (3) their ammonia nitrogen excretion was equal to that of their controls; and (4) their urinary phosphorus to nitrogen ratio was higher (0.119 *vs.* 0.018) than that of

their controls (tumor diet phosphorus to nitrogen ratio was 0.170 and casein diet phosphorus to nitrogen ratio was 0.089).

Recently, F. K. Millar, R. H. Brooks and J. White (*J. Nat. Cancer Inst.* **22,** *685 (1959)*) have extended their studies to examine stimulation of appetite and growth in Sprague-Dawley rats bearing Walker carcinoma 256 or Novikoff rat hepatoma by diets containing (1) 20 per cent or 25 per cent casein, (2) normal rat liver and normal rat hind-leg muscle, (3) crude and defatted Walker carcinoma 256 and Novikoff rat hepatoma, (4) mouse sarcoma 37, mouse adenocarcinoma C3HBA and mouse fibrosarcoma H-38290, and (5) human carcinoma, defatted human carcinoma and human rhabdomyosarcoma.

Interpretation of the data was made difficult by variability in the behavior of tumor-bearing rats even on the control casein diets, variations in the timing and condition of the animals at transfer to a tumor-containing diet, variations in the length of time of the experiments, and differences in the nature of the two experimental tumors.

Rats bearing Walker carcinoma 256 responded equally to diets containing Walker carcinoma 256 or Novikoff rat hepatoma with increases in food intake and weight. Defatted human tumor and defatted Walker carcinoma 256 gave equal growth responses. Normal rat muscle and liver diets produced far smaller gains in intake and weight. Mouse tumor diets fed rats bearing Walker carcinoma 256 produced a response about half of that observed on the Walker carcinoma 256 diet. Diets containing human rhabdomyosarcoma produced weight gains varying from 30 to 90 per cent of gains noted on Walker carcinoma 256 diets. Human carcinoma diet produced excellent responses in three animals but none at all in four.

Rats bearing the transplanted Novikoff hepatoma showed weight-loss reversal and increased food intake and weight gain when fed a diet containing Walker carcinoma 256. When these rats were fed diets containing (1) casein, (2) Walker carcinoma 256 and (3) Novikoff rat hepatoma from the time of tumor implantation, the tumor-containing diets produced greater increases in total body weight and in carcass weight change than did the casein diet. The Walker carcinoma 256 diet produced the largest tumors, and the Novikoff hepatoma the next largest.

The authors are now carrying out fractionation studies on tumor tissue to determine the components responsible for the results described. F. L. Haven and W. R. Bloor (*Fed. Proc.* **14,** *223 (1955)*) described an increase in total body weight of Wistar rats bearing Walker carcinoma 256 fed a diet containing mixed phospholipids ("lipid appetite factor") extracted from Walker carcinoma 256. In a preliminary report, F. K. Millar and J. White (*Proc. Am. Assn. Cancer Res.* **2,** *327 (1958)*) suggested that the residue from alcohol-ether extraction stimulated appetite and weight gain in the rat bearing Walker carcinoma 256.

In their most recent preliminary report, F. K. Millar, J. N. Toal and J. White (*Ibid.* **3,** *43 (1959)*) report that a fraction extracted by boiling water from lipid-free Walker carcinoma 256 resulted in increased weight gain in tumor-bearing animals which had reached a weight plateau. This water soluble fraction contained 10 per cent sodium chloride. When sodium chloride was fed to tumor-bearing rats which had reached a weight plateau, large increases in weight gain and water intake ensued. When sodium chloride in the concentration found in the Walker carcinoma 256 diet (0.9 per cent) was added to the casein diet and that diet fed from the time of tumor implantation, weight gains were comparable to those of tumor-fed rats.

These findings suggest that tumor-bearing rats have a requirement for salt. However, tumor-fed rats performed better than

casein-fed, salt-supplemented rats in more lengthy experiments. Furthermore a water insoluble, salt-free fraction of lipid-free tumor resulted in delayed weight gains when fed from the time of tumor implantation. It is thus suggested that increased salt content may not be the sole explanation for the tumor-diet effects. Studies designed to characterize the unknown factors are now in progress.

CONGENITAL HYPERVITAMINOSIS A

A variety of congenital defects have been produced in rats by the administration of a single large dose of vitamin A on the ninth, tenth or eleventh day of pregnancy.

It has been reported that very high doses of vitamin A given the pregnant rat influence the development of the fetus (*Nutrition Reviews* **15,** *338 (1957)*). The resultant abnormalities include anencephalia (absence of brain), anophthalmos (absence of the eyes) or large, open, protruding eyes, spina bifida (absence of vertebral arches in the spinal column), cleft palate, malformation of the extremities, labial fissures and cataracts. These changes occurred even though the increase in vitamin A per fetus was from 4.15 I.U. in the controls to 10.08 I.U. for those from treated mothers.

Also, deficiencies of vitamin A, riboflavin, folacin, pantothenic acid, vitamin D and vitamin E have been reported to cause various types of congenital deformities (*Nutrition Reviews* **2,** *297 (1944);* **5,** *74, 89 (1947);* **11,** *121 (1953);* **12,** *189, 348 (1954);* **13,** *289 (1955);* **14,** *282 (1956);* **15,** *9, 44 (1957)*).

Recently, a detailed study has been conducted with rats on the influence of a single dose of 75,000 to 150,000 I.U. of vitamin A on the ninth, tenth or eleventh days of pregnancy to determine the nature of the underlying histological changes in the fetus (F. M. Deuschle, J. F. Geiger and J. Warkany, *J. Dent. Res.* **38,** *149 (1959)*). Eight typical specimens with large and protruding eyes were selected from the offspring and serially sectioned. The eyes, although large and lidless, were otherwise almost normal in structure, and the investigators concluded that probably a series of underlying skeletal anomalies caused them to protrude. The lower part of the nasal passage was wider and the upper part narrower in the abnormal rats than in the controls. Where the naso-turbinal and maxillo-turbinal processes approach each other, the nasal passage was almost occluded.

Moreover, these fetuses had a posterior cleft palate and an ankylosis due to a narrow bony ridge between the lateral part of the upper jaw and the lower jaw. There was also a well-developed cartilaginous mass between the upper jaw and lower jaw in an area where there is ordinarily no cartilage. Varying degrees of deficiency were noted in the development of the alveolar processes. In addition, as many as 11 of the 12 molars were missing in some of the specimens. In other cases, the remaining molars were hypoplastic.

Thus the syndrome precipitated in these fetuses by the single administration of a large dose of vitamin A during pregnancy resulted in developmental deficiencies, but only secondarily influenced the eyes because of their relationship to this area. Mesodermal and ectodermal structures were affected and it was presumed that the dental anomalies were caused by the anomalies of the underlying mesodermal tissues.

This hypervitaminosis syndrome could be readily differentiated from that of riboflavin deficiency. In the fetuses produced by riboflavin-deficient rats, only the incisor teeth were affected, with the lowers more

seriously involved than the uppers. In addition, the distal portions of the jaws were reduced in length in riboflavin deficiency whereas the proximal portions were shortened in hypervitaminosis A.

On the basis of present knowledge, no correlation can be drawn concerning pregnancy in women. However, the striking nature of this syndrome and the relative ease with which it was produced would suggest cautious use of large and repeated doses of vitamin A during pregnancy. This caution would seem to be especially pertinent in view of the relatively small difference in total vitamin A content between the carcasses of normal and treated fetuses.

FLUORIDE IN RICKETS

Although the total fluoride taken up in bones is reduced under conditions of rickets, the ratio of fluoride to total bone ash is increased.

Deposition of fluoride in rachitic animals has been reported in two studies both of which pertain to elevated levels of fluoride (J. A. Shultz, *Iowa Agr. Exp. Sta. Am. Rep.*, p. 78 (1936); C. A. Kempf and V. E. Nelson, *Proc. Iowa Acad. Sci.* **48,** *199 (1941)*). It has also been noted that 300 p.p.m. of fluoride reduces the severity of developing rickets by increasing bone density (K. Morgareidge and S. B. Finn, *J. Nutrition* **20,** *75 (1940)*). In addition, the life span of rachitic rats has appeared to be increased when fluorides are made available (S. B. Finn and M. Kramer, *Proc. Soc. Exp. Biol. Med.* **45,** *843 (1940)*).

A more critical evaluation of the deposition of fluoride has been carried out by I. Zipkin, R. C. Likins and F. J. McClure (*J. Nutrition* **67,** *59 (1959)*). In addition to fluoride levels, information was obtained on the fat, ash, calcium and phosphorus contents of the femurs, lower jaws and teeth. The study was especially designed to permit the separation of the effects of rickets per se and inanition.

The experimental diet employed in this study was one which was adequate in calcium (0.44 per cent) and vitamins, but extremely limited in phosphorus (0.018 per cent). Fluoride was added as 2.8 p.p.m. The control diet was supplemented with disodium acid phosphate to provide adequate phosphorus and an adequate calcium to phosphorus ratio (0.437 per cent calcium, 0.559 per cent phosphorus and 3.0 p.p.m. fluoride). Sprague-Dawley rats were divided into three equal groups; group one received the rachitogenic diet and groups two and three received the rachitogenic diet supplemented with disodium acid phosphate. Group two was pair fed to group one and group three was fed ad libitum. The rats were x-rayed after three weeks and sacrificed at six weeks.

Although they received equal quantities of food, the pair-fed non-rachitic rats gained significantly more than their rachitic pair mates. Rats fed ad libitum and receiving the phosphorus-supplemented diet showed weight gains of 2.3 g. per day and a food utilization ratio of 0.27 g. weight gain per g. of diet.

Radiographs revealed that the rats receiving a low phosphorus diet had developed rickets whereas rats given the phosphorus-supplemented diet were normal. Femurs and lower jaws of the rachitic rats weighed much less than those of their normal litter mates, but only small weight differences were observed in the teeth.

The mean concentration of fat of the femurs was greatest in the rachitic, next greatest in the pair-fed rats and least in the ad-libitum fed normals. Fatty contents of the lower jaws followed this same relationship, but no significant differences were seen among the molars and incisors of the three groups. The ash content of the bones

of the rachitic rats was markedly less than that of their non-rachitic pair-fed litter mates, whereas small reductions were seen in the ash concentration of the molars and incisors.

Although the bone ash of the rachitic rats showed a two and one-half-fold increase in fluoride concentration when compared with their non-rachitic pair-fed mates, only half as much total fluoride was detected. No differences in deposition of fluoride were seen in the molars and incisors of the rachitic and their non-rachitic pair-fed litter mates.

The authors emphasize that differences between the rachitic and non-rachitic litter mates represent the effect of rickets, since the dietary intakes were identical. Variations between the pair-fed and ad libitum-fed non-rachitic rats are attributed to inanition. Since no differences were found in the concentrations of ash and fluoride in either the lower jaws or the molars of the two groups of non-rachitic rats (and only small differences were seen in the femurs and incisors), differences in ash and depositions of fluoride were due to the rachitogenic diet rather than to inanition.

The markedly higher relative concentration of fluoride but lower total fluoride in rachitic bones as compared to bones of non-rachitic rats, is due largely to the smaller amount of bone ash present in rachitic bones. It is, however, possible that differences in composition of the bone mineral itself, as seems to be borne out by observed calcium to phosphorus ratios, could play some part in skeletal fluoride deposition. M. Lawrenz and H. H. Mitchell (*J. Nutrition* **22,** *91 (1941)*) found that increasing the phosphorus content from 0.14 to 0.71 per cent in a diet containing 0.71 per cent of calcium also increased the weight of the bones and decreased the relative concentration of fluoride so that the total fluoride content was unchanged.

A markedly lower calcium to phosphorus ratio was observed in the femurs and mandibles of rachitic rats. No changes were seen in either the bones of the two non-rachitic groups or in the molar and incisor teeth of all three groups. In the many other studies which have been carried out on the change of this ratio with the occurrence of rickets, none had the imposed ingestion of fluoride such as featured in this study.

GAMMA-AMINOBUTYRIC ACID AND BRAIN METABOLISM

Brain is the only tissue with large amounts of gamma-aminobutyric acid. Pyridoxine is involved in forming gamma-aminobutyric acid from glutamate. Gamma-aminobutyric acid is an important source of oxidative energy for the brain.

Recent work has shown that the brain is unique in that it contains considerable amounts of gamma-aminobutyric acid (*Nutrition Reviews* **16,** *161 (1958)*). The compound is present almost exclusively in the gray matter. Only traces of the acid have been reported to be present in blood, urine, gastric juice and saliva.

Pyridoxal phosphate is involved in the conversion of glutamic acid to gamma-aminobutyric acid. One of the factore leading to convulsions in pyridoxine-deficient animals and human beings may be the deficiency of the coenzyme (pyridoxine phosphate) for the decarboxylase that converts glutamic acid to gamma-aminobutyric acid. Evidence for the above assumption was provided by the preliminary report of S. R. Dasgupta, E. K. Killam and K. F. Killam (*J. Pharmacol. Exp. Therap.* **122,** *16A (1958)*), which indicated that the topical application of gamma-aminobutyric acid to the cortex of the brain prevented seizures from occurring in a cat injected with thiosemicarbazide. An intravenous injection of pyridoxal had the same effect.

The work of other investigators (*Nutrition Reviews, loc. cit.*) has shown that animals showing seizures following an injection of thiosemicarbazide have a reduced level of cerebral gamma-aminobutyric acid, and that the level of the latter compound can be restored by the administration of pyridoxal.

E. Roberts, M. Rothstein and C. F. Baxter (*Proc. Soc. Exp. Biol. Med.* **97,** *796 (1958)*) from California reported that they found gamma-aminobutyric acid in the nervous system but not in other tissues of fish, reptiles, birds, marsupials and mammals. This compound showed progressive increases at successive developmental stages in the brains of chick embryos, mice, salamanders and frogs.

The above investigators showed that the brain of the mouse is able to convert glutamic acid very rapidly to gamma-aminobutyric acid. This was evident when they injected 35 micrograms of L-glutamic acid, uniformly labeled with C^{14}, intracerebrally in mice. Within fifteen minutes, the ratio of radioactivity of the glutamic acid to that in the gamma-aminobutyric acid had decreased from a value higher than 80 to approximately four. The molar ratio of the amount of the two compounds in the brain was also about four.

When Roberts and co-workers injected 4 mg. of thiosemicarbazide intraperitoneally into mice fifteen minutes prior to the intracerebral injection of the uniformly labeled glutamate, there was a marked reduction in the amount of labeled gamma-aminobutyric acid formed. This is consistent with the work of K. F. Killam and J. A. Bain (*J. Pharmacol. Exp. Therap.* **119,** *255 (1957)*), who found that the semicarbazide inhibited glutamic acid decarboxylase activity in the brain.

G. M. McKhann and D. B. Tower (*Am. J. Physiol.* **196,** *36 (1959)*) have presented *in vitro* evidence that oxidative metabolism of the brain can be maintained at a high level when gamma-aminobutyric acid or succinic semialdehyde is the substrate. They partially exhausted cerebral cortex slices of cats by preincubation for one hour in a phosphate-saline medium without any added substrate. When either glucose, L-glutamic acid, gamma-aminobutyric acid or succinic semialdehyde was added to the medium, the oxygen uptake was maintained (for periods up to two hours) at levels that were significantly higher than for unsupplemented control slices.

Other evidence in the literature led McKhann and Tower (*loc. cit.*) to suggest that during epileptiform seizures (such as those produced by Metrazol), the animal utilizes primarily non-carbohydrates for the oxidative metabolism of the brain. During such times, glutamic acid, which is a likely source of the brain energy during seizures, may be metabolized via gamma-aminobutyric acid. By this means, the glutamate could be brought into the oxidative cycle and thus serve as a source of energy.

A report has appeared on the development of a pyridoxine-dependent deficiency in a child whose mother received large amounts of pyridoxine parenterally during pregnancy (*Nutrition Reviews* **16,** *10 (1958)*). The child required 2 mg. of pyridoxine per day (in addition to that in her diet) in order to remain free of convulsions. This child was studied by McKhann and Tower (*loc. cit.*), who found a reduced cerebral oxygen consumption and respiratory quotient when compared with other subjects. These measurements were rapidly restored to normal following an intravenous injection of pyridoxine. This observation and reports in the literature led the investigators to conclude that "the findings may prove to be consistent with a substrate deficiency due to lack of available gamma-aminobutyrate for support of oxidative metabolism."

G. D. Gammon and R. Gumnit (*Trans. Am. Neur. Assoc. 1957 meeting, p. 57*) have demonstrated that convulsions in mice produced by injections of methoxypyridoxine (a pyridoxine antagonist) can be prevented by the administration of small amounts of

pyridoxine or large amounts of gamma-aminobutyric acid. If this preliminary work is amplified and confirmed, the significance of gamma-aminobutyric acid in both neurochemistry and nutritional investigations will become of tremendous importance.

Summary: The above studies show that gamma-aminobutyric acid occurs in relatively large amounts in the brain and not to any significant extent in any other tissue. They also indicate that the brain can rapidly convert glutamic acid to gamma-aminobutyric acid, and that this compound can maintain the oxidative metabolism of brain slices at a high level for long periods of time. Thus these studies suggest that gamma-aminobutyric acid is a compound of considerable metabolic importance.

ADIPOSE TISSUE AND FAT OXIDATION

Adipose tissue is very active metabolically as shown by its ability to synthesize fats as rapidly as liver. During periods of rapid deposition of body fat, adipose tissue may play a dominant role in fat formation.

It is becoming generally recognized that adipose tissue participates very actively in a number of metabolic reactions. The work of E. Wertheimer and his colleagues has done much to dispel the concept that adipose tissue is inert metabolically. They showed that rats, fasted long enough to exhaust their stores of fat, deposited glycogen in the fat cells with the peak levels occurring seven to eight hours after the animals were fed a high carbohydrate diet (E. Tuerkischer and E. Wertheimer, *J. Physiol.* **104,** *361 (1946)*). As the glycogen disappeared, it was replaced by fat. They suggested that the glycogen initially deposited in the adipose tissue was converted there to triglycerides.

B. Shapiro and E. Wertheimer (*J. Biol. Chem.* **173,** *725 (1948)*) went on to show by *in vitro* studies that adipose tissue may be able to synthesize fat. They used rats fed a stock diet. When they incubated mesenteric and interscapular fat tissue from rats whose food intake was restricted to produce a 20 per cent loss of body weight in five to seven days, the incorporation of deuterium into the fat was at a rate about twice that of the full-fed rats. To enhance the rate of fat synthesis, the starved rats were fed ad libitum for 48 hours prior to sacrifice.

In the above studies, liver slices were more active than adipose tissue in synthesizing fatty acids. Since the amount of adipose tissue in the body is many times the weight of the liver, they suggested that the "adipose tissue may be responsible for a higher percentage of the total quantity of fat synthesized in the body."

The work of F. X. Hausberger, S. W. Milstein and R. J. Rutman (*J. Biol. Chem.* **208,** *431 (1954)*) showed that in *in vitro* experiments, adipose tissue is as active as liver in converting glucose to carbon dioxide. When allowance was made for the large amounts of fat in adipose tissue, it was far more active than liver.

During the past few years, studies have been made of the effect of a number of physiological and dietary factors that influence the activity of adipose tissue and liver. One such study was that by W. F. Perry and H. F. Bowen (*Am. J. Physiol.* **189,** *433 (1957)*), who found that mesenteric fat tissue from adult male rats incorporated more acetate into fatty acids two weeks after the animals had been adrenalectomized than did liver. Adult male rats were given 1 per cent sodium chloride solution as drinking water and a commercial stock diet for either eight or 15 days postoperatively. At the end of this time, the liver and mesenteric adipose tissue were removed.

The adipose tissue was incubated with C^{14} labeled acetate. It was found that the adipose tissue from the rats which had been

adrenalectomized for one week produced fatty acids with the same number of counts as the control tissue. On the other hand, the adipose tissue from the animals that had been adrenalectomized for two weeks contained fatty acids having twice the counts of the preceding tissues. These results suggested that the adipose tissue from animals that had been adrenalectomized for two weeks incorporated a larger amount of acetate into fatty acids than did the control tissue. The incorporation of acetate was also greater than that in tissues from animals which had been adrenalectomized for only one week.

When the adipose tissue was incubated with a buffered solution of C^{14} labeled octanoic acid, the tissues from the controls and the animals that had been adrenalectomized for two weeks produced the same amount of labeled carbon dioxide. Although the metabolic rate was the same in the two tissues, the adipose tissue from the control animals incorporated almost three times as much octanoic acid into fat as did the tissues from the adrenalectomized rats.

The livers from the control rats incorporated approximately three times as much labeled acetate into fatty acids as did the livers from the animals adrenalectomized for one week. The livers from the rats that had been adrenalectomized for two weeks produced one and one-half times as much acetoacetic acid as did the livers from the control rats. The latter study was carried out with labeled octanoic acid.

The above studies lend additional support to the thesis that the adipose tissue is very active metabolically. However, the observations on the effect of adrenalectomy may be open to some question since the adrenalectomized animals progressively lost body fat following the operation, and this may have influenced the results.

A number of years ago, H. C. Stoerk and C. C. Porter (*Proc. Soc. Exp. Biol. Med.* **74,** *65 (1950)*) showed that adrenalectomized rats, when properly treated, showed an increase in their body fat stores following adrenalectomy. They used adult male rats whose food intake had been restricted to about one-third their requirement for eight days. At the end of this time, the animals were adrenalectomized and then permitted food ad libitum. One per cent sodium chloride solution was the drinking water.

Five days after adrenalectomy, the animals were killed. At this time, the testicular fat pad of the adrenalectomized rats was only slightly lighter than that of the sham operated controls (0.287 vs. 0.318 g. per cent of final body weight). These weights represented a two-fold increase over the testicular fat pads removed from another group of animals that at the time of adrenalectomy had lost as much weight as those that were operated upon. Carcass analyses carried out on another series of rats indicated that the weight of the testicular fat depots reflected the total body fat content.

The work of Stoerk and Porter (*loc. cit.*) suggests that the adipose tissue in the adrenalectomized rats has the ability to lay down fat even when no therapeutic measure other than saline solution is used. It would be interesting to determine whether conditions could be devised whereby animals that had not been starved prior to the operation would show the same effect.

More recently, experimental work continues to confirm the high physiological activity of the adipose tissue. A few questions are, however, being raised as to the specific sequence of events. For instance, J. P. Miller, J. A. D. Cooper and S. Freeman (*Proc. Soc. Exp. Biol. Med.* **95,** *817 (1957)*) found changes in the concentration of glucose and fat in adipose tissue which did not appear to support the thesis proposed by Tuerkischer and Wertheimer (*loc. cit.*).

Miller, Cooper and Freeman fasted adult rats until they lost 20 per cent of their initial body weight. This required about 48 hours. The rats were then refed a high carbohydrate diet ad libitum to increase the metabolic activity of the adipose tissue. Animals were

sacrificed at intervals following the initiation of the refeeding.

The epididymal fat pads removed during the first six hours after refeeding showed an increase in lipid content. The level rose from 69 per cent in the zero hour samples to 81 per cent in the six-hour samples. During this time, there was a slight reduction in the glucose content of the adipose tissue (0.14 per cent at zero hour; 0.09 per cent at six hours). From the sixth through the forty-eighth hour, the lipid level in the tissue gradually fell from the high of 81 per cent to 59 per cent. During this time, the glucose level progressively increased from 0.09 per cent to 1.03 per cent.

When the above data are plotted, it becomes evident that the adipose tissue from the rats that were refed has a lipid level which inversely mirrors the changes in glucose concentration. No lag period is evident during which the glucose level reaches a peak prior to the initiation of lipid synthesis.

On the basis of the carbon dioxide produced during incubation of the adipose tissue removed from the rats during refeeding, there is a continuous increase in metabolic activity of that tissue throughout the 48 hours of refeeding. The adipose tissue of the refed rats produced more carbon dioxide than the tissue from the unstarved controls.

The rate of incorporation of labeled acetate into fatty acids by the incubated adipose tissue was depressed in the tissues removed from the rats during the first 18 hours of refeeding. By the twenty-fourth hour, the rate was the same as that in the unstarved control rats, while at 48 hours, the rate was one and one-half times that of the controls.

These studies emphasize the high metabolic activity of the adipose tissue and suggest something of the role it plays in bioenergetics. It has been firmly established that adipose tissue is able to synthesize fats. Since there is so much adipose tissue in the body, it may play a more important role in this respect than the liver, especially on those occasions when the body is rapidly laying down fat.

The generally accepted thesis is that adipose tissue first accumulates glycogen and then converts the latter to triglycerides. Recent evidence might suggest that the formation of these two substances occurs almost simultaneously. With the tremendous amount of attention being given to adipose tissue, this problem should soon be resolved.

AMINO ACID DEFICIENCIES IN CASEIN

A new order of growth limiting amino acids is indicated for casein as follows: methionine; threonine; tryptophan, isoleucine, leucine, histidine, valine and phenylalanine; lysine; nonessential amino acids.

As early as 1915, T. B. Osborne and L. B. Mendel (*J. Biol. Chem.* **20,** *351 (1915)*) established that the adequacy of casein as a source of dietary protein was determined by its content of the sulfur-containing amino acids. It is also known that casein is low in both tryptophan (W. A. Krehl, P. S. Sarma and C. A. Elvehjem, *Ibid.* **162,** *403 (1946)*) and threonine (W. H. Griffith and M. F. Nawrocki, *Fed. Proc.* **7,** *288 (1948)*). However, other than for methionine, threonine and tryptophan, it is difficult to find information regarding the sequence in which the amino acids of casein become limiting for the growth of rats.

Since it is necessary to know the exact sequence in which the amino acids in a protein become limiting for growth in order to study amino acid balance and imbalance in detail, a series of critical experiments with 6.0 per cent of casein as the sole source of dietary protein has been undertaken by A. E. Harper (*J. Nutrition* **67,** *109 (1959)*). Supplementation with different amino acids, in amounts

sufficient to bring the growth levels to normal, provided the method of assay.

Male weanling rats of the Sprague-Dawley strain, 21 days old and weighing from 40 to 50 g. were used. Each group consisted of five rats. The experiments were of two weeks duration. Percentage composition of the basal diet was as follows: casein, 6.0; carbohydrate, 84.6; corn oil, 5.0; salts, 4.0; and vitamins. In many of the experiments, two series of diets were used, one containing sucrose, the other containing dextrin, as the carbohydrate. This provided a simple method of obtaining measurements with two levels of protein intake. Amino acid supplements were compensated for by decreases in the carbohydrate fraction of the diet.

As was to be expected, methionine proved to be the most limiting amino acid in casein. Threonine, not tryptophan, was the second amino acid to become limiting for the growth of rats fed this diet. Combined supplementation with methionine and threonine caused a substantial growth effect, regardless of the type of dietary carbohydrate, and further addition of tryptophan produced no further growth. Supplementation of the diet containing methionine and threonine with various combinations of histidine, lysine and tryptophan (the amino acids calculated to be most limiting after methionine and threonine) gave no further growth response.

Since the sequence of amino acid deficiencies determined experimentally failed to correspond to the calculated sequence, a series of mixtures of amino acids, each lacking only arginine and one of the other indispensable amino acids, were tested as supplements to both the sucrose and the dextrin diets containing 6 per cent of casein plus 0.4 per cent of DL-methionine and 0.5 per cent of DL-threonine. The results were that amino acid mixtures lacking tryptophan, isoleucine, leucine, histidine, valine or phenylalanine all failed to stimulate growth. Only when all six of these amino acids were added to the basal diet was the rate of gain greater than that of the negative control group, and only after further supplementation with lysine did it significantly exceed that of the basal group.

Even when the seven amino acids were included at levels designed to make the levels of essential amino acids at least equivalent to those of a diet containing 12 per cent of casein supplemented with 0.2 per cent of L-cystine, the growth rate was well below that obtained with a complete purified diet.

From this experiment, the author concludes that tryptophan, isoleucine, leucine, histidine, valine and phenylalanine were approximately equally limiting after methionine and threonine, and that lysine was only slightly less limiting than the other six.

When the opposite procedure (that of adding amino acids singly or in small groups) was used, the rate of gain again did not exceed that of the basal group unless tryptophan, isoleucine, leucine, histidine, phenylalanine and valine were all added to the diet. Comparable, but somewhat higher values, were obtained in one experiment with diets containing 9 per cent casein supplemented with the various amino acid mixtures.

In a further attempt to determine why the rate of gain had been suboptimal for rats fed on diets containing a low level of protein supplemented with supposedly adequate levels of essential amino acids, the requirement of *nonessential* amino acids under these conditions was studied. A group of rats was fed a diet containing 6 per cent of casein plus a sufficient amount of the nonessential amino acids to give levels of the L-amino acids equal to these in a diet containing 12 per cent of casein supplemented with 0.2 per cent of L-cystine, together with sufficient glutamic acid to equalize the nitrogen intake. Growth in this group was nearly equal to that of a group receiving an adequate quantity of intact casein.

When a mixture containing 6 per cent casein and supplements of methionine, threonine, tryptophan, isoleucine, leucine, histidine, valine and phenylalanine was fed to rats, additional glutamic acid did not stimulate growth unless lysine or lysine and argi-

nine were added. Lysine stimulated growth by itself, but only when arginine, lysine and glutamic acid were all provided was growth comparable to that obtained with an adequate purified diet. A mixture of DL-aspartic acid, DL-alanine and glycine was as effective as glutamic acid and an increase in the level of the nonessential amino acid mixture from 1.9 to 4 per cent of the diet was without effect.

The conclusion from these experiments is that the essential amino acids in a diet containing a low level of casein as the only source of protein, become limiting in the order already mentioned, but that the nonessential amino acids are just slightly less limiting than lysine and must be provided before the full response to supplements of arginine and lysine can be obtained.

W. C. Rose, L. C. Smith, M. Womack and M. Shane (*J. Biol. Chem.* **181,** *307 (1949)*) and others have demonstrated a response to nitrogenous compounds other than essential amino acids in rats fed diets containing only the nonessential amino acids. It is possible that the effect of glutamic acid is an indirect one in which an excess of glutamic acid reduces the amount of the essential acids being broken down by catabolic reactions.

A. E. Harper is of the opinion that three factors could be responsible for the generally observed deviations between the sequence of amino acid deficiencies determined experimentally and those calculated: (1) the amino acid analyses for casein may be in error, (2) all of the amino acids in casein may not be readily available, and (3) the estimated requirements for the rat may be high. The author favors the last factor since it is difficult to determine accurately the minimum requirements for maximum growth due to the nature of the growth curve and the influence of other factors affecting food consumption.

However, now that purified laboratory diets have been much improved and more is known of dietary interrelationships, reinvestigation of the requirements can and should be made.

FRUITS, SEROTONIN, AND CATECHOL AMINES

Ingestion of bananas leads to increased excretion of 5-hydroxyindole acetic acid and of catechol amines, a fact of concern in the diagnoses of carcinoid and pheochromocytoma by measuring the urinary excretion of these substances.

The carcinoid syndrome (*Nutrition Reviews* **15,** *156 (1957)*) is an example of a rare disease which promises to provide us with unforeseen information about biochemical interrelationships, both cerebral and somatic. In this disorder a malignant carcinoid (argentaffin) tumor of the bowel metastasizes to the liver. Here the metastatic nodules release excessive quantities of serotonin directly into the systemic circulation. This hormone (5-hydroxytryptamine) produces a syndrome well characterized by A. Thorson, G. Biörck, G. Björkman and J. Waldenström (*Am. Heart J.* **47,** *795 (1954)*). Serotonin in large quantities produces cutaneous flushing alternating with cyanosis, intestinal cramping with diarrhea, bronchoconstriction with asthma and, often pulmonic valvular stenosis.

Serotonin is formed from tryptophan which is hydroxylated to 5-hydroxytryptophan, then decarboxylated to 5-hydroxytryptamine. This active compound is degraded by mono-amino oxidase to 5-indolehydroxy acetic acid (5 HIAA). A method for determination of this substance in the urine was described by S. Undenfriend, E. Titus and H. Weissbach (*J. Biol. Chem.* **216,** *499 (1955)*). Normal persons excrete 2 to 8 mg. of 5 HIAA, but persons with metastatic malignant carcinoid in the liver may excrete as much as 350 mg. daily.

Other studies of serotonin have shown its biochemical interrelations. A deficiency of pyridoxine impairs the rate of decarboxylation of 5-hydroxytryptophan to 5-hydroxytryptamine, slowing the rate of formation of serotonin (*Nutrition Reviews* **16,** *85* (*1958*)). Administration of iproniazide interferes with mono-amino oxidase, which converts serotonin to the inactive 5 HIAA (M. I. Gluckman, E. R. Hart and A. S. Marrazzi, *Science* **126,** *448* (*1957*)). Although large quantities of tryptophan given to patients with the carcinoid syndrome will be taken up by the tumor (*Nutrition Reviews* **16,** *223* (*1958*)), average dietary variations probably have little effect on urinary excretion of 5 HIAA by normal man.

These and other isolated details have been integrated by P. A. Shore, S. L. Silver, and B. B. Brodie (*Science* **122,** *284* (*1955*)) and by B. B. Brodie (*Modern Medicine* **16,** *No. 15, p. 69* (*1958*)), who described an interrelationship between serotonin, reserpine and lysergic acid. According to theory, nervous reactions of mammals can be classified as *ergotropic* or excitatory and as *trophotropic* or recuperative. Norepinephrine is the hormone which mediates the ergotropic phase while serotonin mediates the trophotropic phase. In view of this theory, actions of many drugs such as tranquilizers and central nervous system excitors can be explained.

Thus reserpine induces release of increased quantities of serotonin in the hypothalamus, where its effect is to lower blood pressure and reduce excitement. Conversely, the hypertensive hallucinatory excitement induced by lysergic acid diethylamide is due to release of norepinephrine in the hypothalamus. It is apparent that serotonin has a degree of importance which extends far beyond that of the carcinoid syndrome.

In 1958, J. A. Anderson, M. R. Ziegler and D. Doeden (*Science* **127,** *236* (*1958*)) reported the accidental discovery that monkeys fed bananas excreted much more 5 HIAA in their urine as a result of this feeding. Part of this effect was blocked by iproniazid (because it inhibits mono-amino oxidase). Subsequently, T. P. Waalkes *et al.* (*Ibid.* **127,** *648* (*1958*)) analyzed bananas and found that the average banana contains about 3.5 mg. of serotonin and considerable amounts of pressor amines (presumably norepinephrine). An average banana weighs about 150 g.

D. W. Woolley (*Nature* **180,** *630* (*1957*)) has speculated on the phylogenetic possibilities of serotonin being the animal counterpart of auxin, a plant growth hormone. Indeed, auxin (3-indoletryptamine) differs from serotonin (5-hydroxy-3-indoletryptamine) only in the absence of an OH group at carbon 5. This leads one to ponder why bananas should contain serotonin at all.

Recently, A. Barbeau and L. J. Wilkoff reported studies of the urinary excretion of 5 HIAA and of catechol amines by a healthy young man whose diet was controlled (*Canad. Med. Assn. J.* **80,** *717* (*1959*)). When he was fed a simple diet free of fresh fruits or vegetables he excreted normal amounts of 5 HIAA (method of Sjoerdsma) and no detectable catechol amines (strip of rabbit aorta—method of Helmer). When he ate 11 bananas daily (average 100 g. each) for three days, his excretion of 5 HIAA increased six to ten fold. At the same time he excreted 100 micrograms of pressor substances daily in his urine. These values would be interpreted as indicative of clinical disease if his diet were unknown. When he ate only three bananas daily he excreted nearly normal quantities of 5 HIAA and of pressor amines. A diet containing grapefruit and oranges had no effect on the urinary excretion of either substance.

Since the biologic test employed can give positive results with a number of compounds (5 HIAA, adrenaline, nor-adrenaline, dopamine and other catechols), the authors tested the effect of regitine added to the perfusion chamber. This prevented the contraction of the aortic strip, hence the substance presumably was a catechol amine.

In a further effort to identify these amines, the authors repeated their study, feeding bananas to three more subjects. Chromatographic analyses of samples of urine disclosed that at least part of the active substance which appeared in the urine after ingestion of bananas was norepinephrine.

The authors concluded that a diet containing large amounts of bananas (five or six daily) can produce false positive reactions in the tests used to diagnose the carcinoid syndrome and pheochromocytoma. They stressed the fact that the subjects who ate large amounts of bananas had no change in pulse or blood pressure.

This investigation may lead to new clues which can expand our knowledge of such diverse topics as cerebral metabolism and plant growth processes. From unexpected sources come keys to future discoveries.

THE CHEMICAL AND PHYSICAL EFFECTS OF ASCORBIC ACID DEFICIENCY ON THE TISSUES DURING WOUND HEALING

Guinea pigs were used in studies of ascorbic acid deficiency. Animals were fed different levels of ascorbic acid or none and tissue levels were determined by chemical analysis. Strength of healed abdominal wounds was determined in all groups.

The role of ascorbic acid in the healing of wounds has been evaluated by studies of the effect of a deficiency of this vitamin on the chemistry and cellular response of tissues subjected to surgical wounds. Recent reviews (*Nutrition Reviews* **14,** *152* (*1956*), **15,** *114* (*1957*) and **16,** *218* (*1958*)) disclose that ascorbic acid deficiency reduces the rate of collagen synthesis. This is important in promoting adequate tissue repair. The cause of the failure to synthesize collagen appears to be a primary inability to convert proline to hydroxyproline.

A. F. Abt, S. Von Schuching and J. H. Roe (*Bull. Johns Hopkins Hospital* **104,** *163* (*1959*)) have studied the relation of dietary intake of ascorbic acid to its concentration in the tissues of guinea pigs with surgically induced wounds.

The guinea pigs used were young males weighing 220 to 250 g. The strength of the healed midline abdominal incision was determined by noting the pressure needed to rupture the wound by inflation of the abdominal cavity. The chemical determinations were performed on blood, adrenals and tissue samples of skin and muscle near the wound as well as on distant abdominal skin and muscle.

Two types of vitamin C deficient diet were used, a rabbit chow and a semi-synthetic diet prepared for guinea pigs according to M. E. Reid and G. M. Briggs (*J. Nutrition* **51,** *341* (*1953*)). A solution containing 60 mg. of ascorbic acid was given each animal daily by dropper while comparing the two diets. Under these conditions there were no obvious differences in ascorbic acid levels in blood or tissues and no significant variations in the pressures necessary to produce wound rupture between the two diet groups.

Different dietary intakes of ascorbic acid, however, produced definite effects on wound healing and tissue levels of the vitamin. When ascorbic acid was not fed, the pressure producing wound disruption was markedly reduced and tissue levels of the vitamin were diminished. With an increase in dietary intake, however, the concentration in skin, muscle and adrenals increased. Significantly, the skin and muscle of the wound had a higher level of ascorbic acid than did the distant abdominal skin and muscle, except at low levels of intake. Unoperated animals showed ascorbic acid concentrations comparable to those of the distant skin and muscle in operated animals, indicating that operative stress did not appear to influence tissue levels of the vitamin.

The ascorbic acid in the skin of an ascorbic

acid deficient group at the time of operation averaged 7.4 mg. per 100 g. At the time of sacrifice, the wound skin averaged 2.9 mg. per 100 g. and the adrenals averaged 13.7 mg. per 100 g. Blood ascorbic acid was about 0.2 mg. per cent in one deficient group on the Reid and Briggs diet, and 0.6 mg. per cent in a deficient group fed rabbit chow. By the time of sacrifice the blood levels had diminished to about 0.1 mg. per cent. In the group receiving 30 mg. of ascorbic acid daily, the blood concentration averaged 1.4 mg. per cent at operation and 0.68 mg. at sacrifice. Abdominal skin at operation averaged 12.1 mg. per 100 g. and wound skin at sacrifice 17.0 mg. per 100 g. The adrenals averaged 132 mg. of ascorbic acid per 100 g.

A second series of animals was fed a semi-synthetic diet supplemented with 60 mg. of ascorbic acid daily for a preliminary period of eight days. Following this, the animals were divided into two groups, one with no ascorbic acid and the other receiving 90 mg. per day. After receiving these diets for ten days, the abdominal operation was performed and both groups were sacrificed at three, six, nine and 12 days post-operatively. In both groups the pressure required for rupture of the wounds increased as time passed. In all instances the pressure measurement for the deficient group was less than that of the controls.

The tissue levels of ascorbic acid varied from group to group at the time of operation. For example, in the deficient animals the abdominal muscle samples ranged from 3.6 to 4.9 mg. of ascorbic acid per 100 g., and at sacrifice the wound muscle varied from 1.3 to 1.6 mg. per 100 g. There was no change in the ascorbic acid content as the period increased between operation and sacrifice. Similarly, in control animals the ascorbic acid content of abdominal muscle ranged from 5.4 to 7.7 mg. per 100 g. at the time of operation. At sacrifice, the values ranged from 7.9 to 10.5 mg. per 100 g. with no relation to the number of days between operation and sacrifice.

Blood levels at sacrifice ranged from 0.12 mg. to 0.3 mg. per cent in the deficient group, unrelated to the post-operative interval, while in the control group blood levels diminished from 1.85 mg. to 0.72 mg. per cent from three to 12 days after operation. Adrenal ascorbic acid ranged between 13.7 and 29.8 mg. per 100 g. in the deficient groups and between 151 and 182 mg. per 100 g. in the controls, again without relation to time following the operation.

Summary: Several points of interest appeared in the results of these studies of ascorbic acid and repair of wounds in guinea pigs. One was the increase in the concentration of ascorbic acid in skin and muscle of the wound as compared with distant tissue during ascorbic acid administration. Also, a steady increase in the strength of the healing wounds in scorbutic animals was noted, despite a 22 day deficiency of ascorbic acid. However this is probably not a very severe deficiency state, even though the ascorbic acid level in wound skin, blood, adrenal and muscle was relatively low as determined by analysis.

Comparison of a commercial rabbit food with a semi-synthetic diet showed little difference in efficiency of wound repair or tissue levels of ascorbic acid when the vitamin was fed at 60 mg. per day. On the other hand, the feeding of different levels of the vitamin was reflected by differences in the pressure of rupture of the healing wound as well as in differences in blood and tissue concentrations.

The fact that unoperated animals showed ascorbic acid concentrations equivalent to those in operated animals in areas away from the wound, indicated that surgical stress did not affect the amounts of ascorbic acid in these animals.

The source and metabolic significance of the ascorbic acid which accumulates in the healing wound is of interest. Therefore, the proposed studies of Abt, Von Schuching and Roe using radioactive ascorbic acid will be an important sequel to this study.

NOTES

Letter to the Editor

Dear Sir:

I note in the issue of *Nutrition Reviews* for June, 1959, an article on "Sorbitol and Vitamin B_{12} Absorption" (*Nutrition Reviews* **17,** *188 (1959)*). The impression gained is that the sorbitol enhances the absorption of vitamin B_{12} in rats and in normal human subjects.

This is not always so, if the dose of vitamin B_{12} is small, according to others who have worked on this problem (B. A. Cooper, *Nature* **182,** *647 (1958)*; H. C. Heinrich, R. Skibbe and M. Staak, *Zeitschrift fur Naturforschung* **14b,** *42 (1959)*; H. C. Heinrich, *Klinische Wochenschrift* **37,** *525 (1959)*).

Sincerely yours,
W. B. CASTLE, M.D.
Director, Thorndike Memorial Laboratory
Boston City Hospital

Symposium on Lipids

The papers presented at the Sixth Annual Symposium of the Institute for Metabolic Research were published as the final number of the *American Journal of Clinical Nutrition* for 1958 (*Am. J. Clin. Nutrition* **6,** *583–687 (1958)*). This symposium, called the Deuel Conference on Lipids, after the late Harry Deuel, dealt with the chemistry, biochemistry and metabolism of lipids. Publication editor for this number was Dr. L. W. Kinsell, whose introduction outlines the background of the symposia.

The symposium, as published, consists of 24 papers by some 30 authors whose names are closely linked with recent developments in the chemistry and biochemistry of lipids. The papers, which range from a lipid-oriented parody of Longfellow's "Hiawatha" by Hugh Sinclair to a discussion of gas-liquid chromatography by A. T. James, will be of value, both in themselves and as sources of references. Chemical methods, absorption, transport, fatty acid deficiency, cholesterol relationships and essential fatty acid metabolism are all discussed.

It is very valuable to have the proceedings of these small symposia available. One can only regret that space does not permit publication of the discussions.

Recent Books

Facts on the Major Killing and Crippling Diseases in the United States Today. Compiled in 1959 by The National Health Education Committee, Inc., 135 East 42nd Street, New York 17, New York. Price $5.25.

Physiopathologie des Aminoaciduries, by J. Kruh. Article in *Sem. Hôp. Paris* **35,** page 862 (4 Mars 1959). This is an excellent summary respecting a number of syndromes not often discussed in the United States.

Methods of Biochemical Analysis. Edited by David Glick, Professor of Physiological Chemistry, University of Minnesota. Interscience Publishers, New York. Price $9.50. Pp. 353.

The Role of Nutrition Education in Combatting Food Fads. A 37-page booklet of four papers presented by representatives of industry, education, the Food and Drug Administration and the Federal Trade Commission as a part of a symposium on nutrition research and education held under the joint sponsorship of the Northern California Section, Institute of Food Technologists and The Nutrition Foundation, Inc. Published by The Nutrition Foundation, Inc., 99 Park Avenue, New York 16, New York.

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